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VITAL ACTIVITY OF *LACTUCA SATIVA* AND SOIL MICROORGANISMS UNDER FLUORESCENT FILMS

Productivity of *Lactuca sativa*, variety Moskovsky parnikovy growing in protected cultivation under fluorescent film luminescent with maximum of 615 nanometers is studied in dynamics. Change of conditions of plant cultivation by fluorescent film promotes accelerated growth, development and productivity improvement of *Lactuca sativa* relative to plants growing under unmodified film. Increase in productivity of *Lactuca sativa* in 1.5 times under fluorescent films is determined by increase in ratio IAA/ABA and decrease in content of AA in plants, and also conjugated with change of enzymatic activity of aboriginal soil microflora.

Key words: *Lactuca sativa, protected cultivation, fluorescent films, productivity, hormones, ascorbic acid, soil microflora.*

In the conditions of increase in the number of population, environmental pollution and deficiency of food supply, the problem of crop maximizing in order to get ecologically pure product is paramount [1]. One of decisions of this problem is creation of effective artificial ecosystems, including open ecosystems of protected cultivation [2] where solar energy is used, and unfavorable conditions for plants are changed at the expense of modification of greenhouses polymeric covering [3, 4].

In agricultural practice the use of fluorescent films luminescent in visible spectrum due to UV radiation absorption by luminophor loaded into their structure recently has been found [3–12]. The use of such films as coverings of greenhouses leads to increase in plants productivity by 10–90 % [13–22].

Plants productivity is connected with soil fertility in which enzyme catalase and heterotrophic microorganisms participating in decomposition of organic compounds play considerable role [23]. However connection between increase in productivity and soil microorganisms activity in plant cultivation in greenhouse with fluorescent films is not ascertained.

The research purpose. Investigation of of the influence of low intensity red luminescent radiation generated by fluorescent films on morphogenesis, accumulation of photosynthetic pigments (PSP), ascorbic acid (AA) synthesis, level of endogenous hormones of *Lactuca sativa* and soil microorganisms activity.

Methods. Three-year tests were conducted at agrobiological research station of Tomsk State Pedagogical University by detection of soil microbiological activity, morphometric and biochemical characteristics of plants cultivated in greenhouses, covered with fluorescent (experiment) and not modified (control) polyethylene films. Arch-form greenhouses measuring 1x1 m, 0.6 m by height, as the soil – mixture of equal quantities of chernozem, humus and peat were used.

Polymeric compositions for films were manufactured by method of dry mixture of HDPE granules of mark 15303-020 with powdery luminophor by technology [24]. Samples of sleeve films of 120 microns thickness were prepared by method of blown extrusion. As the additive for manufacturing of fluorescent film luminophor on the basis of a complex of europium nitrate with 1,10-phenantrolin – Eu $(NO_3)_3$ x 2Phen was used [5–8]. Photophysical characteristics of films were estimated in the spectra from spectrometer «AvaSpec 2048» (Avantes, the Netherlands) and acoustooptical spectrometer «Quartz 3102B» by techniques [25] (Table 1).

The objects of researches were plants *Lactuca sativa*, variety "Moscowsky parnikovy". Seeds were sowed into soil in 1st June and cultivated within 40 days. Measurement of plant growth parameters were carried out every 10 days. The leaf area of *Lactuca sativa* was calculated by paper-weight method. The wet mass and dry weight of plants were determined on analytical balance with accuracy of 0.1 mg. For the detection of dry weight plants were dried up to constant weight at temperature 103–105 °C.

Table 1

Structure and some photophysical properties of fluorescent (experiment) and not modified (control) polyethylene films

Type and code number of the film		Control	Experiment
Type of used luminophor		–	Eu $(NO_3)_3$ x 2Phen
The basic maximum in luminescence spectrum, nm		–	615
Quantity of luminophor, % of masses		0.0	0.1
Intensity of a luminescence, relative units		0.0	104.8
Transmission of electromagnetic radiation, % in ranges of nm *	290–330	59.1	51
	320–400	65.2	59
	380–710	76.0	73
Integral optical transmission, %		94.7	93.6

* The note: ranges in accordance with the State Standard 10354.

The testing of PSP content was carried out on spectrometers AvaSpec-2048FT-2-SPU (Avantes, the Netherlands) in 100 % acetone extracts of plant mate-

rial, and calculated by Holma's formula [26].

Extraction and detection of AA content was determined by method [27].

The extraction of endogenous hormones was carried out from sample of fresh plant material fixed by liquid nitrogen and extracted by 70 % ethanol [28]. For isolation of free IAA and ABA an extract was evaporated to water rest and extracted by diethyl ether at pH=3.0 [29]. The separation of free IAA and ABA was implemented by means of thin-layer chromatography on Silufol UV-254 strips ("Kavalier", Czechia) in the system of solvents: diethyl ether – chloroform – acetic acid (100:100:1, by volume). For the identification of substances on chromatogram standard samples of IAA and ABA ("Sigma-Aldrich") were used. The quantitative determination of phytohormones was carried out by solid-phase immunoenzyme method [30] with use of reagents of domestic manufacture ("Farmhiminvest", Russia). IAA and ABA activity was determined by extension degree of cuts coleoptiles of *Triticum vulgare* L., variety Novosibirskaya-29 relating to control in 2 % solution of sucrose with the use of dilution of samples and standard substances (calibration) [28].

Measurements of morphometric parameters and biochemical tests were performed on 30 plants in 5 replications. The specialized package "Statistic for Windows" (the program "Excel") was used for statistical treatment of experimental results. The estimation of reliability of researches results was conducted with 95 % confidence (significance level – 0.05). In tables and figures arithmetic mean with two-sided confidence interval is presented.

The abundance of a microflora was studied by an example of the heterotrophic bacteria growing on beef-extract agar (BEA). Dynamics of the number of microflora was determined in 4–5 days. Soil samples after careful mixing was sterile selected from 5–7 different places and combined into one, samples were analyzed immediately after sampling. Microflora was isolated by method of limit dilution on selective agar mediums in 5 replications. The sowed dishes were temperature-controlled during 5–7 days at 30–35 °C, and then a number of colonies was calculated [31].

Catalase activity was determined by gasometric method (by volume of precipitated oxygen), by speed measurement of hydrogen peroxide decomposition in its interaction with soil [31].

Results and discussion. Results of studies have shown various plants growth answers depending on the use as a covering of a greenhouse film. In experiment growth inhibition of *Lactuca sativa* hypocotyl in comparison with control was noted, by 9 days hypocotyl length of experimental plants was less than control in 2.3 times (Fig. 1).

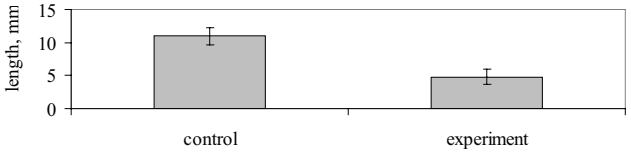

Fig. 1. Hypocotyl length of *Lactuca sativa*, cultivated with fluorescent (experiment) and not modified (control) films (9 days)

Follows more active plant development accompanied by faster formation of leaf blade was observed in experiment as compared with control. Significant increase in leaf number, shoot length and leaf area in 1.08, 1.54 and 1.50 accordingly times were determined at 19-day-old experimental plants relating to control, and at 29-day-old plants – in 1.15, 1.43 and 1.31 times accordingly (Fig. 2–4).

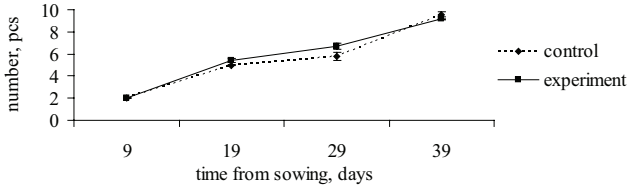

Fig. 2. Dynamics of *Lactuca sativa* leaf number, cultivated with fluorescent (experiment) and not modified (control) films

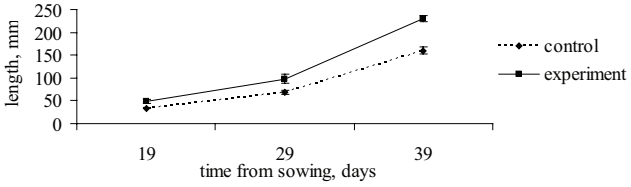

Fig. 3. Dynamics of *Lactuca sativa* shoot length cultivated with fluorescent (experiment) and not modified (control) films

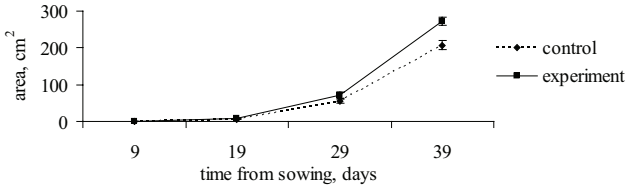

Fig. 4. Dynamics of *Lactuca sativa* leaf area cultivated with fluorescent (experiment) and not modified (control) films

Dynamics of wet mass and dry weight of plants were conjugated with changes of habitus (Fig. 5, 6).

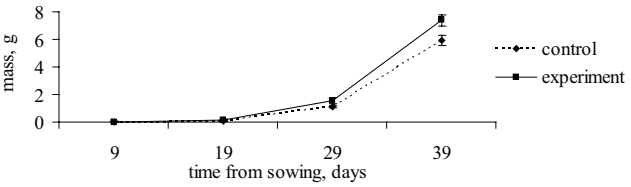

Fig. 5. Dynamics of wet mass *Lactuca sativa* cultivated with fluorescent (experiment) and not modified (control) films

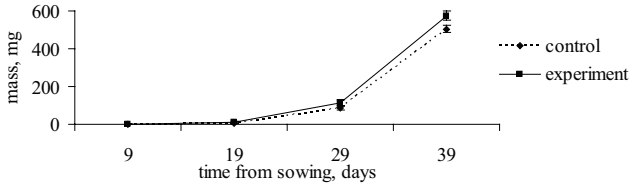

Fig. 6. Dynamics of *Lactuca sativa* dry weight cultivated with fluorescent (experiment) and not modified (control) films

Dynamics of plant wet mass was correlated with increase in leaf blade area (r=0.99), and dynamics of plant dry weight – leaf number (r=0.87). The maximal differences were noted at 9, 19 and 29 days: increase in wet mass of experimental plants relating to control in 1.23, 1.52 and 1.42 times accordingly was noticed, dry weight –in 1.15, 1.44 and 1.28 times accordingly.

Change of *Lactuca sativa* productivity in control and experiment was conjugated with dynamics of AA accumulation in plant leaf and growth substances (Fig. 7, Table 2).

In experimental plants as compared to control the increased IAA level and minimum ABA content were noticed at 9–19 days of vegetation. Follows the decrease in IAA content of experimental plants was connected with its expenditure to formation of reproductive organs. The smaller size of control plants of salad in comparison with experimental, possibly, was mediated by higher endogenous ABA content. It's possible to suppose, that level of IAA and ABA, involved in transduction system of a light signal, depends on luminescent radiation of fluorescent films and changes the work of salad regulatory systems [32]. It leads to acceleration of plant growth and development under fluorescent films and was reflected in their productivity.

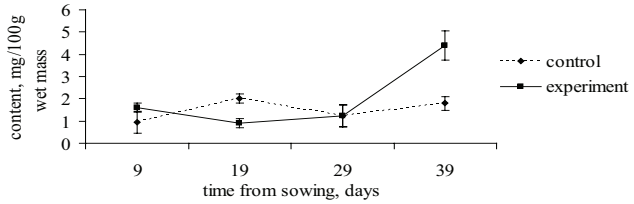

Fig. 7. Dynamics of *Lactuca sativa* AA level cultivated with fluorescent (experiment) and not modified (control) films

Table 2

Content of endogenous IAA and ABA of Lactuca sativa L. variety Moscowsky parnikovy, cultivated in Tomsk region during the period from June, 1st till July, 11th with not modified (control) and fluorescent (experiment) films

Age of plants, days	The content of endogenous hormones, a ng/plant			
	Free IAA		Free ABA	
	The control	Experiment	The control	Experiment
9	Traces	2.93 ± 0.29	0.88 ± 0.35	0.44 ± 0.02
19	0.19 ± 0.01	0.47 ± 0.09	0.26 ± 0.05	0.18 ± 0.03
29	0.47 ± 0.08	Traces	0.84 ± 0.20	0.62 ± 0.15
39	Traces	1.17 ± 0.29	6.17 ± 0.93	0.31 ± 0.07

Minimum AA content in experiment relating to control was noted at the moment of maximum difference of their productivity (at 19–20 days). Decrease in AA level in 2.2 times in experiment is connected with its expenditure on process of growth that was confirmed by the literary data [33]. It's known that AA takes part in biochemical transformations underlying of growth, though low AA content promotes activation of plant growth.

The increase in *Lactuca sativa* productivity in protected cultivation with fluorescent film was not accompanied by changes in level of PSP accumulation in plant leaf and indicated that plants inside greenhouse got optimum quantity of PAR for normal process of photosynthesis. The result received by us is confirmed by the data presented in [5, 13, 17, 20, 22] about optimal work of plant photosynthetic apparatus and optimal accumulation of pigments by plants in plastids at cultivation with fluorescent films. The sunlight passed through the fluorescent film improved plants productivity, most probably, due to change of low-energy reactions responsible for individual plant development [34–35], specified by photophysical films properties (Table 1) and change of growth substances level (Table 2)

Increase in productivity of *Lactuca sativa* in experiment relating to control was conjugated with changing of soil microflora (Fig. 8, 9).

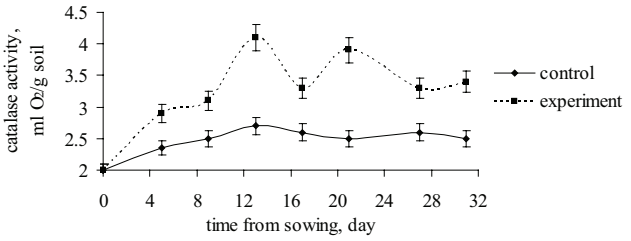

Fig. 8. Dynamics of catalase activity in soil under fluorescent (experiment) and not modified (control) films

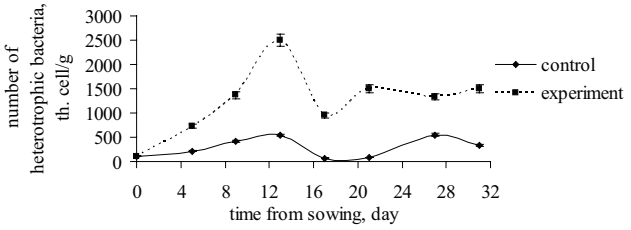

Fig. 9. Dynamics of number heterotrophic bacteria in soil under fluorescent (experiment) and not modified (control) films

In samples of soil under fluorescent film increase in catalase activity in 1.2–1.6 times relating to control was noticed throughout all experiments (Fig. 8). Oxygen formation of control and experimental samples was characterized by two maxima at 9–13 and 21 days. Maximal concentration of oxygen 3.9–4.1 ml/g was noted during the given periods in experimental soil.

The abundance level of soil heterotrophic microflora was conjugated with catalase activity in soil in experiment and control (r=0.85 and r=0.52 accordingly). Original number of heterotrophic bacteria in experimental and control soils was 100–110 thousand cell/g (Fig. 9). During the experiment increased number of studied microorganisms in soil under fluorescent film to comparison with soil under unmodified film was noted. In experiment maximal increase in number of heterotrophic microflora in 13–19 times relating to control was noted at 17–21 days.

Such intensification of soil aboriginal microflora activity promoted accelerated growth and rootage development of experimental plants in relation to control in initial period of vegetation (during 3 weeks). Dynamics of catalase activity and dynamics of increase in number of heterotrophic bacteria were correlated with the change of dry matter and wet mass of salad roots (r=0.80, r=0.74 and r=0.78, r=0.68 accordingly) under fluorescent film.

In the literary data indicating determinative significance of photophysical properties of films used for protected cultivation, in change of soil microbiological parameters, and growth and rootage development of plants are presented [36]. In our researches sunlight influence on soil microbiological parameters, conjugated with growth and development of plant roots, and their productivity as a whole, were defined by specific property of fluorescent films – luminescent radiation (Fig. 10).

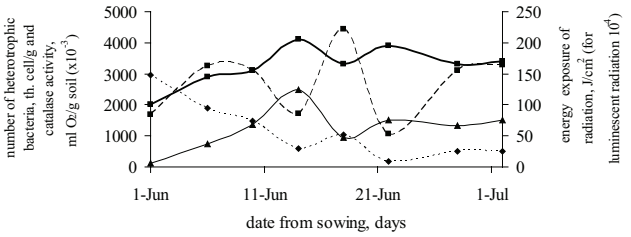

Fig. 10. Dynamics of (—■—) catalase activity and (—●—) number of soil heterotrophic bacteria, (– - ■ - -) UV sun radiation and (· · · ◆ · · ·) luminescent radiation of fluorescent film at cultivation of *Lactuca sativa* L. variety «Moscowsky parnikovy» in Tomsk region during the period from June, 1st till July, 11th, 2010

Presented results showed that dynamics of soil aboriginal microflora activity was determined by dynamics of luminescent radiation of fluorescent film. Peaks of increase in number of heterotrophic bacteria and catalase activity in soil were fitted at 3–5 days after maximal exposition of luminescent radiation of fluorescent films (UV radiation exciting luminescence of luminophor in film).

The conclusion. The use as a covering of greenhouse fluorescent film absorbing a part of UV radiation and transforming it in to RL with a maximum 615 nanometers, promoted increase in productivity of *Lactuca sativa*. It occurred at the expense of the influence of fluorescent film luminescent radiation on reduction of AA content, increase in ratio IAA/ABA in plants, and it was conjugated with change of enzymatic activity of soil aboriginal microflora.

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ЖИЗНЕДЕЯТЕЛЬНОСТЬ *LACTUCA SATIVA* И МИКРООРГАНИЗМОВ ПОЧВЫ ПОД ФЛУОРЕСЦЕНТНЫМИ ПЛЕНКАМИ

Изучали в онтогенезе продуктивность *Lactuca sativa* сорта Московский парниковый при выращивании в защищенном грунте под флуоресцентной пленкой, люминесцирующей с максимумом 615 нм. Изменение условий выращивания растений флуоресцентной пленкой способствуют ускоренному росту, развитию и повышению продуктивности *Lactuca sativa* относительно растений, выращенных под немодифицированной пленкой. Повышение продуктивности *Lactuca sativa* в 1.5 раза под флуоресцентными пленками определяется увеличение соотношения ИУК/АБК и уменьшением содержания АК в растениях, а также сопряжено с изменением ферментативной активности аборигенной почвенной микрофлоры.

Ключевые слова: *салат, защищенный грунт, флуоресцентные пленки, продуктивность, гормоны, аскорбиновая кислота, микрофлора почвы.*

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